Direct Solvent Extraction of Sympathomimetic Amines from Biological Samples

1 Introduction

Sympathomimetic amines (SMAs) are generally a class of synthetic phenethylamine-derived drugs often generically referred to as “amphetamines”. Almost all of these compounds show some degree of stimulant effects, but a wide variety of additional structure-dependent pharmacological effects can be seen in various compounds. These include pure stimulants (amphetamine and methamphetamine), decongestants (phenylpropanolamine and pseudoephedrine), anorexics (phentermine and fenfluramine), and hallucinogens (mescaline, one of the few relevant naturally occurring SMAs). Over the last few decades there has been particular interest in and concern over the widespread illicit use of various “designer” SMAs with combined stimulant and hallucinogenic properties. The “type specimen” of this class is 3,4-methylenedioxy-methamphetamine (MDMA or “ecstasy”), which was originally developed for possible use as an adjunct drug in psychotherapy, but now is one of the most widely used illicit drugs in teenage and young adult populations. Chemists in clandestine drug laboratories have developed a wide array of related compounds, including thioalkyl- and halogen-containing analogues, in attempts to stay ahead of drug scheduling regulations. In approximately 2010, several new designer amphetamines including methylone, mephedrone, and 3,4-methylenedioxypyrovalerone began appearing on the U.S. abused drug scene as “bath salts”.

2 Scope

This procedure allows for screening and confirmation of a wide range of SMAs, and is currently validated for quantitation of amphetamine, methamphetamine, ephedrine / pseudoephedrine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), methylenedioxyethylamphetamine (MDEA), methylone, mephedrone and 3,4-methylenedioxypyrovalerone (MDPV). With appropriate validation, it may be used for quantitation of other detected SMAs.

3 Principle

Biological specimens are qualitatively assayed and/or quantitated for SMAs. Specimens are mixed with an internal standard (normally a mixture of six deuterated SMAs), adjusted to a basic pH, and extracted with hexane. (When quantitating bath salt compounds, a mixture of deuterated bath salt compounds is used as the internal standard mixture.) The hexane is removed, acidified to prevent evaporation of volatile SMAs, and taken to dryness. The resulting residue is reconstituted in 10/90 methanol/water and analyzed by LC-ESI-MS with data dependant MS^2 and MS^3. MS^3 detection is

included because some SMAs yield uninformative MS^2 spectra with limited information content. The extraction procedure is derived from work by Sadeghipour and Veuthey. The chromatographic and mass spectral procedures and parameters were developed in-house.

4 Specimens

This procedure uses a biological sample such as: blood, serum, plasma, urine, gastric contents, vitreous humor, or a prepared tissue homogenate. When available, 0.5 mL of biological fluid or 1.0 g of tissue homogenate (1:1) is used in the assay. In instances where specimen volume is altered (e.g. to improve sensitivity or account for limited specimen volume), appropriate modifications to this procedure may be made.

5 Equipment/Materials/Reagents

Guidance for the preparation of reagents may be found in the *Preparation of Chemical Reagents* standard operating procedure (Tox 103).

a. 16x100 mm screw-top tubes with Teflon-lined caps

b. 12x75 mm culture tubes with polypropylene snap-tops

c. Acetonitrile (Optima grade or better)

d. Formic Acid (Puriss grade or better)

e. Hexane (UV grade or better)

f. Hydrochloric acid (ACS grade or better)

g. Methanol (Optima grade or better)

h. Sodium hydroxide (ACS grade or better)

i. Water (Deionized and Optima or better grade)

j. 4% Sodium hydroxide
Dissolve 2 g sodium hydroxide in 50 mL deionized water. Store in plastic at room temperature. Stable for at least 6 months.

k. Methanol:Hydrochloric Acid (4:1 v:v)
Mix 20 mL methanol with 5 mL hydrochloric acid. Store in glass at room temperature. Stable for at least 1 month.

l. Methanol:Water (10:90 v:v)
Mix 5 mL methanol with 45 mL water (both Optima grade or better). Store in glass at room temperature. Stable for at least 1 year.

m. 0.1% Formic acid in acetonitrile
Vacuum filter 500 mL acetonitrile through a 5 µm PTFE membrane and mix with 0.5 mL formic acid. Store in glass at room temperature. Stable for 2 months.

n. 0.1% Formic acid in water
Vacuum filter 500 mL water (Optima grade or better) through a 5 µm PTFE membrane and mix with 0.5 mL formic acid. Store in glass at room temperature. Stable for 2 months.

o. Vortex mixer, Rotator and Centrifuge

p. Evaporator with nitrogen

q. Routine laboratory supplies, including disposable pipettes, wooden sticks, test tube racks, graduated cylinders, etc.

r. Liquid Chromatograph-Ion Trap Mass Spectrometer

s. HPLC Column (Xterra C18, 2.1 x 150 mm, 5 µm dp, with a 2.1 x 7.5 mm guard column; or equivalent)

6 Standards and Controls

a. Internal Standard Stock Solutions (0.1 mg/mL) may be purchased from Cerilliant or other approved supplier. Stability and storage conditions are determined by the manufacturer.

d_5-Amphetamine	d_5-MDEA
d_5-Methamphetamine	d_3-Mephedrone
d_5-MDA	d_3-Methylone
d_5-MDMA	d_8-MDPV

b. Internal Standard Working Solution (2 µg/mL each of common components):
Combine 0.5 mL each of the d_3-ephedrine, d_5-amphetamine, d_5-methamphetamine, d_5-MDA, d_5-MDMA, and d_5-MDEA stock solutions in a 25 mL volumetric flask. Add 2

mL methanol and bring to the mark with water (both Optima grade or better). Store in glass at <0ºC. Stable for at least 2 years.

c. Bath Salts Internal Standard Working Solution (2 µg/mL each of d_3-mephedrone, d_3-methylone, and d_8-MDPV):
Combine 0.5 mL each of the d_3-mephedrone, d_3-methylone, and d_8-MDPV stock solutions in a 25 mL volumetric flask. Add 2 mL methanol and bring to the mark with water (both Optima grade or better). Store in glass at <0ºC. Stable for at least 2 years.

d. Standard Stock Solutions (1 mg/mL) may be purchased from Cerilliant (typically used for calibrators) and from Lipomed (typically used for controls) or another approved supplier. Stability and storage conditions are determined by the manufacturer.

Ephedrine	MDMA
MBDB (N-methylbenzodioxazolylbutanamine, N-methyl-1-3,4-methylenedioxy-phenyl)-2-butanamine)	MDEA
Amphetamine	Mephedrone
Methamphetamine	Methylone
MDA	MDPV

e. Amine Mixture-6 (250 µg/mL each component):
A methanol solution containing amphetamine, methamphetamine, phentermine, MDA, MDMA, and MDEA purchased from Cerilliant or another approved vendor. Stability and storage conditions are determined by the manufacturer.

f. Column Performance Evaluation Mix (1 µg/mL each component):
Combine 25 µL each of the MBDB and ephedrine stock solutions with 100 µL of the Amine Mixture-6 in a 25 mL volumetric flask. Add 2.4 mL methanol and bring to the mark with water (both Optima grade or better). Stable for at least 2 years. A 10 µL portion of this solution is analyzed before each day's samples, in order to confirm acceptable instrument performance.

g. Control Working Solution (1 µg/mL each component):
Mix 50 µL each of the ephedrine, amphetamine, methamphetamine, MDA, MDMA, and MDEA stock solutions in a 50 mL volumetric flask. Add 9.9 mL methanol and bring to the mark with water (both Optima grade or better). Store in glass at <0ºC. Stable for at least 1 year. Note: Once verified, this solution can be parsed out into small containers for freezing.

h. Bath Salts Control Working Solution (1 µg/mL each component):
Mix 50 µL each of the mephedrone, methylone, and MDPV stock solutions in a 50 mL volumetric flask. Add 9.9 mL methanol and bring to the mark with water (both Optima

grade or better). Store in glass at <0ºC. Stable for at least 1 year. Note: Once verified, this solution can be parsed out into small containers for freezing.

i. Calibration Working Solution #1 (5 µg/mL each component):
Mix 250 µL each of the ephedrine, amphetamine, methamphetamine, MDA, MDMA, and MDEA stock solutions in a 50 mL volumetric flask. Add 8.5 mL methanol and bring to the mark with water (both Optima grade or better). Store in glass at <0ºC. Stable for at least 1 year. Note: Once verified, this solution can be parsed out into small containers for freezing.

j. Calibration Working Solution #2 (0.5 µg/mL each component):
Mix 25 µL each of the ephedrine, amphetamine, methamphetamine, MDA, MDMA, and MDEA stock solutions in a 50 mL volumetric flask. Add 9.9 mL methanol and bring to the mark with water (both Optima grade or better). Store in glass at <0ºC. Stable for at least 1 year. Note: Once verified, this solution can be parsed out into small containers for freezing.

k. Bath Salts Calibration Working Solution #3 (5 µg/mL each component):
Mix 250 µL each of the mephedrone, methylone and MDPV stock solutions in a 50 mL volumetric flask. Add 9.25 mL methanol and bring to the mark with water (both Optima grade or better). Store in glass at <0ºC. Stable for at least 1 year. Note: Once verified, this solution can be parsed out into small containers for freezing.

l. Bath Salts Calibration Working Solution #4 (0.5 µg/mL each component):
Mix 25 µL each of the mephedrone, methylone and MDPV stock solutions in a 50 mL volumetric flask. Add 9.9 mL methanol and bring to the mark with water (both Optima grade or better). Store in glass at <0ºC. Stable for at least 1 year. Note: Once verified, this solution can be parsed out into small containers for freezing.

m. Negative Control Blood and/or Urine:
Purchased from Diagnostics Products Corporation, UTAK Laboratories, Inc., Cliniqa, or prepared in-house from an appropriate blank specimen. Blood and urine will be stored refrigerated, frozen or obtained fresh. Stability determined by manufacturer. A Negative Control will be extracted and analyzed with every assay. When possible, the negative control will be matrix matched.

n. Quantitative Positive Control Blood:
This is normally prepared in-house as per the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101), but may be purchased from an appropriate vendor as needed. Storage and stability determined by manufacturer. Normally prepared at concentrations of 60 and 400 ng/mL by adding 30 and 200 µL of the Control Working Solution to 0.5 mL samples of Negative Control Blood on the day of extraction. Other levels and matrices may be used as circumstances dictate.

o. Bath Salts Quantitative Control Blood:
This is normally prepared in-house as per the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101), but may be purchased from an appropriate vendor as needed. Storage and stability determined by manufacturer. Normally prepared at concentrations of 60 and 400 ng/mL by adding 30 and 200 µL of the Bath Salts Control Working Solution to 0.5 mL samples of Negative Control Blood on the day of extraction. Other levels and matrices may be used as circumstances dictate.

p. Qualitative Positive Control Blood or Urine:
This is normally prepared in-house, but may be purchased from an appropriate vendor as needed. Storage and stability determined by manufacturer. Normally prepared at a concentration of 200 ng/mL by spiking a 0.5 mL portion of negative control matrix with 100 µL of the Control Working Solution and the Bath Salts Control Working Solution. Other levels may be used as circumstances dictate. Additionally, deuterated analog internal standards serve as a qualitative positive control for each individual specimen.

7 Calibration

This procedure may be used quantitatively via construction of a multi-point calibration curve with equal weighting for the analyte(s) of interest following the *Guideline for Toxicological Quantitations* standard operating procedure (Tox 101). Table 1 shows typical concentrations and volumes for blood calibrators.

Table 1: Typical Blood Calibrator Preparation

Cal Level (ng/mL)	Blood Volume (mL)	Calibrator Working Solution #1 Volume (µL)	Calibrator Working Solution #2 Volume (µL)
25	0.5	0	25
50	0.45	0	50
75	0.45	0	75
100	0.4	0	100
250	0.5	25	0
500	0.45	50	0
750	0.45	75	0

Table 2: Bath Salts Blood Calibrator Preparation

Cal Level (ng/mL)	Blood Volume (mL)	Bath Salts Calibrator Working Solution #3 Volume (µL)	Bath Salts Calibrator Working Solution #4 Volume (µL)
25	0.5	0	25
50	0.45	0	50
75	0.45	0	75
100	0.4	0	100
250	0.5	25	0
500	0.45	50	0
750	0.45	75	0

8 Sampling

Not applicable.

9 Procedure

Appendix 1 contains an abbreviated version of this procedure. This form may be used at the bench by the examiner or chemist performing the procedure.

a. To a properly labeled 16x100 mm screw-top tube add 0.5 mL of biological fluid or 1 g of a prepared tissue homogenate (1:1 in water). Also prepare Negative and Positive Controls as described in Section 6. For quantitation, prepare calibrators as described in Section 7, and prepare case samples in duplicate.

b. Add 50 µL of the Internal Standards Working Solution, resulting in a concentration of 200 ng/mL for each internal standard. For bath salts quantitations, add 50 µL of the Bath Salts Internal Standard Working Solution.

c. Add 0.2 mL of 4% sodium hydroxide to each sample and vortex briefly.

d. Add 2 mL of hexane to each tube and extract for 20 minutes on a rotator. Centrifuge 10 minutes at a minimum of 3000 rpm. Use a wooden stick to break up any emulsions that develop, and recentrifuge if necessary.

e. Transfer organic (top) layer to a 12x75 mm culture tube.

f. Add 0.1 mL of 4:1 methanol:hydrochloric acid and vortex briefly.

g. Evaporate to dryness under a gentle stream of nitrogen at approximately 40°C.

h. Reconstitute the dried residue in 0.1 mL of 10:90 methanol:water.

i. Analyze by 10 µL by LC-MS-ESI with data dependent tandem MS (DDS) with the conditions given below (Sections 10.1 and 10.2).

10 Instrumental Conditions

Appendix 2 contains a checklist of method parameters that should be used to verify proper instrumental conditions prior to analysis of case samples.

10.1 Liquid Chromatograph Parameters (Shimadzu Prominence, or equivalent)

Mobile Phase Compositions	Flow Parameters			Column Parameters	
1: 0.1% formic acid in acetonitrile	total flow	0.3 mL/min		type	C18
	time (min)	%1	%2	length	150 mm
2: 0.1% formic acid in water	0	7.5	92.5	internal diameter	2.1 mm
	5	7.5	92.5	particle size	5 µm
	20	60	40	temperature	40°C
	23	60	40	guard length	7.5 mm
	28	7.5	92.5	guard ID	2.1 mm
	32	7.5	92.5		
	total time	32 min			

10.2 Mass Spectrometer Parameters with DDS (Thermo / Finnigan LTQ, or equivalent)

Source Parameters			
Mode: Electrospray	Spray Voltage: +5 kV		Capillary Temperature: 250ºC
Sheath Gas: 25 (arb units)	Aux Gas: 10 (arb units)		Sweep Gas: 0 (arb units)
All other source parameters are set through the tuning process. See the appropriate IOSS standard operating procedure for details.			
1 Segment with 3 Scan Events			
Event #1	full scan m/z 125-350		
Event #2*	MS/MS data dependent scan		collision energy: 70 (rel)
	precursor: most intense ion from event #1, excluding m/z 141, 155, 169, 181, 185, 199, 211, 213 and 284, threshold = 1000 counts		
	isolation width: 2.0 AMU		scan range: software control
Event #3*	MS^3 data dependent scan		collision energy: 70 (rel)
	precursor: most intense neutral loss of 17, 18, 31, or 45 observed in event #2, threshold = 1000 counts		
	isolation width: 2.0 AMU		scan range: software control
Dynamic Exclusion Enabled for Data Dependent Scanning			
repeat count	10	repeat duration	30 seconds
exclusion list size	25	exclusion duration	30 seconds
expiration count	5	expiration threshold	s/n<5
exclusion width	-1 to +2 amu		

*These events can also be limited to one mass or several masses for targeted analysis.

11 Decision Criteria

11.1 Batch Acceptance Criteria

No analytes of interest should be detected in the Negative Control. For this purpose, analytes of interest are defined as those analytes that will be reported for this batch.

All intended analytes should be present in the Positive Control. Each Quantitative Positive Control shall quantitate within ±20% of the target value. See the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101) for more information.

11.2 Sample Acceptance Criteria

11.2.1 Chromatography

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. In order to be determined acceptable, a chromatographic peak in an unknown sample should compare favorably to a chromatographic peak of the same analyte in a known sample analyzed on the same system in the same or subsequent analytical runs. Additionally, the following two criteria should be met.

11.2.2 Retention Time

The retention time of the peak should be within ± 5% of the retention time (relative or absolute, as appropriate) obtained from injection of a reference standard, calibrator, or Positive Control.

11.2.3 Signal-to-Noise

To justify the existence of a peak, its baseline signal to peak-to-peak noise ratio should exceed 3. Further, the baseline signal for the peak of interest should be at least 10 fold greater than that for any observed peak at similar retention time in a Negative Control or blank injected just prior to the sample.

11.2.2 Mass Spectrometry (for Data Dependent Scanning Analysis)

The mass spectrum of the analyte of interest should match that of a reference standard or an extracted Positive Control within a reasonable degree of scientific certainty. See the *Guidelines for Comparison of Mass Spectra* standard operating procedure (Tox 104) for general criteria. Mass spectral fragments of all SMAs tested in validation and found to extract via this procedure are listed in Table 3. In most circumstances the MS^2 and MS^3 (when present) spectra in an unknown sample should have all the same significant ions as the spectra of the known analyte in a contemporaneously analyzed standard, control, or calibrator, and should not have any significant ions not present in the known spectrum. Additionally, for any compound in Table 3 with two primary ions listed for a given spectral level, the intensity ratio for those ions should meet the requirements given in the Tox 104 standard operating procedure.

Table 3: Mass Spectrometry Data for Sympathomimetic Amines (precursor ions in bold type when multiple ions listed)

Compound Name	Precursor from Full Scan MS	Primary MS^2 Product Ion(s)	Primary MS^3 Product Ions(s)
Amphetamine	136	119	91
Cathinone	150	132	117
Methamphetamine	150	119	91
Phentermine	150	133	91
Phenylpropanol amine	152	134	117
Ethylamphetamine	164	119	91
Methcathinone	164	146	131
(pseudo)Ephedrine	166	148	133, 117
PMA	166	149	121
Benzylpiperazine	177	91, 85	not triggered
Propylamphetamine	178	119, 91	not triggered
Mephedrone	178	160	not triggered
MDA	180	163	135, 133
PMMA	180	149	121
2C-H	182	165	150
Dimethoxypheneth ylamine	182	165	150
4-MTA	182	165	137, 117
BDB	194	177	147, 133
MDMA	194	163	135, 133
Dimethoxy-amphetamine	196	179	151
Chlorophenyl piperazine	**197**, 199	154	not triggered
Methylone	208	190, 160	not triggered
MBDB	208	177	135
MDDMA	208	163	135, 133
MDEA	208	163	135, 133
DOM	210	193	178,156
Mescaline	212	195	180
DOET	224	207	192, 179
Trimethoxy-amphetamine	226	209	194, 181
Trifluoromethyl	231	188	not triggered

Compound Name	Precursor from Full Scan MS	Primary MS^2 Product Ion(s)	Primary MS^3 Product Ions(s)
phenylpiperazine			
Fenfluramine	232	**187**,159	159
Methylphenidate	234	84	not triggered
2-CT-2	242	225	210,164
2-CT-4	256	239	197
2-CT-7	256	239	224, 197, 164
2C-B	**260**, 262	243	228, 164
DOB	**274**, 276	257	229, 178
MDPV	276	205, 175, 126	not triggered
2C-I	308	291	276, 164

12 Calculations

See the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101) for acceptable practices in calculating quantitative results.

13 Uncertainty of Measurement

The critical sources of measurement uncertainty in this procedure include:

- historical random uncertainty of repeated measurements
- accuracy of the pipette or balance used to deliver the sample
- accuracy of the pipette used to deliver the calibrators
- uncertainty in the concentration of the calibration standards
- precision of the delivery of internal standard

When quantitative results are included in an FBI Laboratory report, the measurement uncertainty will be estimated and reported following the *Chemistry Unit Procedures for Estimating Uncertainty in Reported Quantitative Measurements* standard operating procedure (CUQA 13). Information used to derive uncertainty measurements will be tracked in an electronic database.

14 Limitations

a. Method Performance Parameters (Blood and Urine):
LOD = Limit of Detection; LLOQ = Lower Limit of Quantitation

Compound	LOD in Blood (ng/mL)	LOD in Urine (ng/mL)	LLOQ (ng/mL)	Linear Range (ng/mL)	Accuracy (average % bias)	Precision (average % intermediate)
Amphetamine	5	5	10*	10-750	+5.7	4.8
Methamphetamine	5	5	25	25-750	+0.6	4.2
(pseudo)Ephedrine	10	10	10*	10-750	+1.9	3.5
MDA	5	5	25	25-750	+5.1	4.5
MDMA	5	5	25	25-750	+3.9	3.9
MDEA	5	5	25	25-750	-0.7	5.2
Methylone	25	10	25	25-750	-10.9%	3.7
Mephedrone	25	10	25	25-750	-11.5%	3.8
MDPV	25	2	25	25-750	-3.4%	2.0

*Although amphetamine and pseudo(ephedrine) have been validated to an LOQ of 10 ng/mL, for routine analysis, curves will be analyzed down to 25 ng/mL. Therefore, any results below the curve will be reported as less than 25 ng/mL (less than the lowest calibrator.)

b. Interferences: Grossly decomposed or putrefied samples may affect both detection and quantitation limits. High levels of PMMA may interfere with accurate quantitation of MDA, and high levels of BDB may interfere with accurate quantitation of MDMA. The following compound pairs will be difficult or impossible to differentiate by this procedure: ephedrine and pseudoephedrine; 4-chlorophenylpiperazine and 3-chlorophenylpiperazine.

c. Other Considerations: At concentrations below approximately 25 ng/mL, some analytes may show a strong signal in full MS extracted ion chromatograms, but show no tandem MS signal due to the interaction of data dependent scan conditions and dynamic exclusion parameters. If there is good reason to suspect that this has happened, the questioned sample should be reinjected with scan event #2 changed to target only the ion(s) of interest and dynamic exclusion disabled. This procedure is not able to distinguish different optical isomers of SMAs, and cannot distinguish between the diastereomeric compounds ephedrine and pseudoephedrine. The following phenethylamine-group compounds were tested and found to not be extractable via this procedure: HMA (hydroxymethoxyamphetamine), HHMA (hydroxymethamphetamine), HMMA (hydroxymethoxymethamphetamine), and salbutamol.

15 Safety

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

16 References

Sadeghipour, F. and Veuthey, J., *Journal of Chromatography A*, v. 787 (1997), pp. 137-143

Baselt, R.C., *Disposition of Toxic Drugs and Chemicals in Man*, 7th ed., Biomedical Publications: Foster City, California, 2004.

Guidelines for Toxicological Quantitations (Tox 101); FBI Laboratory Chemistry Unit – Toxicology Subunit SOP Manual.

Chemistry Unit Procedures for Estimating Uncertainty in Reported Quantitative Measurements (CUQA 13); FBI Laboratory Chemistry Unit Quality Assurance and Operations Manual.

Preparation of Chemical Reagents (Tox 103); FBI Laboratory Chemistry Unit – Toxicology Subunit SOP Manual.

Guidelines for Comparison of Mass Spectra (Tox 104); FBI Laboratory Chemistry Unit – Toxicology Subunit SOP Manual.

FBI Laboratory Chemistry Unit – Instrument Operation and Support Subunit SOP Manual.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
2	03/08/12	Removed hair to its own SOP (Tox 428) and updated all affected sections. Added methylone, mephedrone and MDPV (i.e., bath salts) as validated analytes and updated all affected sections. Updated LC column brand in 5s. Allowed for frozen storage of blood and urine in 6dd. Changed Control Working Solution concentration from 10 to 1 µg/mL in 6 x and updated Quantitative Positive Control Levels in 6ee from 200 ng/mL to 60 and 400 ng/mL. Updated Chromatography Decision Criteria in 11.1.
3	07/09/14	In Section 6, combined stock solutions into tables, included Lipomed as a source for controls, and included option to freeze prepared calibrators and controls in small vials after verification. In Section 7, specified equal weighting for the calibration curve. In Section 9.a, specified duplicate analyses for quantitation. In 9.d, added recentrifuge step. In Section 9.i, specified injection volume. In Section 10.2, included option of targeted analysis. Added Section 11.1 and renumbered subsequent sections. In 14.a, explained that in routine analysis, calibration for amphetamine and (pseudo)ephedrine will be calibrated down to 25 ng/mL, despite the validated LOQ of 10 ng/mL. Added calibrator and control preparation to Appendix 1 and removed reagent instructions. Reformatted Appendix 2 to include all pertinent instrumental parameters.

Approval

Redacted - Signatures on File

Appendix 1: Abbreviated version of the SMA procedure for bench use.

Redacted - Form on File

Appendix 2: Instrumentation parameters checklist for the SMA procedure.

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